MPI RESEARCH
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Analytical Report

PFOA and PFOS Analysis of Deer Serum Samples by LC/MS/MS

MPI Report No. L0019345

Testing Laboratory

MPI Research, Inc.
3058 Research Drive
State College, PA 16801

Requester/Project Manager

Dena Haverland
Dalton Utilities
PO BOX 869
Dalton, GA 30722
Phone: 706-529-1010

1 Introduction

Results are reported for the analysis of deer serum samples received at MPI Research from Dalton Utilities. The MPI Research study number assigned to the project is L0019345. Table I lists the target analytes quantitated for the samples.

Table I. Target Analytes for Quantitation

Compound Name	Acronym
Perfluorooctanoic Acid	C8 Acid or PFOA
Perfluorooctanesulfonate	C8 Sulfonate or PFOS

2 Sample Receipt

Two samples were received from Dena Haverland at Dalton Utilities for this study. The samples were collected on October 02, 2009. The samples arrived on October 06, 2009 via Fedex and were logged in under MPI Research login number L0019345. The shipment was received frozen on dry ice. The samples were stored frozen at approximately -80°C from receipt until analysis. Chain-of-custody information is presented in Attachment A.

3 Methods - Analytical and Preparatory

3.1 Serum Sample Preparation

3.1.1. Measure 1 mL of serum sample into a 50 mL disposable centrifuge tube and fortify, if appropriate. Add 0.2 mL of a 100 ng/mL WIS for a final concentration of 0.5 ng/mL.

3.1.2 Add water to sample for a final volume of 20 mL. Cap tightly and vortex for ~1 minute.

3.1.3 Transfer 1 mL of the sample using a disposable pipette into 15 mL disposable centrifuge tubes. Add 5 mL of ACN and shake for ~20 minutes on a wrist action shaker.

3.1.4 Centrifuge tubes at ~3000 rpm for ~ 5 minutes. Carefully decant supernatant into a 50 mL disposable centrifuge tube and add 35 mL of water.

3.1.5 Place the unconditioned SPE columns on the vacuum manifold. Condition the SPE columns by passing ~ 10 mL of methanol through the column followed by ~ 5 mL of water. The washes may be pulled through the SPE column using vacuum at a flow rate of ~1 drop/sec or may be allowed to pass through the column unaided. Discard all washes. Do not allow the column to dry.

3.1.6 Load the sample onto a conditioned SPE column . Discard the eluate. Any analyte residues will be trapped on the SPE column at this point.

3.1.7 Elute with 2 mL of methanol. Collect 2 mL of elute into a graduated 15 mL centrifuge tube.

3.2 Sample Analysis by LC/MS/MS

In High Pressure Liquid Chromatography (HPLC), an aliquot of extract is injected and passed through a liquid-phase chromatographic column. Based on the affinity of the analyte for the stationary phase in the column relative to the liquid mobile phase, the analyte is retained for a characteristic amount of time. Following HPLC separation, mass spectrometry provides a rapid and accurate means for analyzing a wide range of organic compounds. Molecules are ionized, fragmented, and detected. The ions characteristic of the compounds are observed and quantitated against external calibration standards.

An HP1100 system interfaced to an Applied Biosystems API 4000 LC/MS/MS was used to analyze the sample extracts for quantitation. A gradient elution through a Phenomenex Luna 3µ C8(2) Mercury, 20 x 4.0 mm column was used for separation.

The following gradient was performed:

Mobile Phase (A): 2mM Ammonium Acetate in Water
Mobile Phase (B): Methanol

Time	%A	%B
0.0	90	10
0.5	90	10
2.0	10	90
5.0	10	90
5.1	0	100
6.0	0	100
6.1	90	10
10.0	90	10

The following parameters were used for operation of the mass spectrometer:

Parameter	Setting
Ionization Mode	Electrospray
Polarity	Negative
Transitions Monitored	413→369 (PFOA)
	499→80 (PFOS)
	415→370 (Internal Std. ^{13}C PFOA (m+2))
	503→80 (Internal Std. ^{13}C PFOS (m+4))
Gas Temperature	450°C

4 Analysis by LCMSMS

4.1 Calibration

For the serum sample analysis, a 6-point calibration curve was analyzed throughout the analytical sequence for PFOA and PFOS. The calibration points were prepared at 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 ng/mL (ppb) each containing 1.0 ng/mL ^{13}C-PFOA (m+2) and ^{13}C-PFOS (m+4).

The ratio of the analyte concentration to the IS concentration versus the ratio of the analyte instrument response (area) to the IS response (area) was plotted for each point. Using linear regression with 1/x weighting, the slope, y-intercept and coefficient of determination (r^2) were determined. A calibration curve is acceptable if $r^2 \geq 0.985$.

For the results reported here, calibration criteria were met. The calibration curves are included in the raw data in Attachment C.

4.2 Laboratory Control Spikes

Laboratory control spikes in the analytical set were prepared during each extraction set by adding a known concentration of the analyte to deer serum controls. Laboratory control spikes are used to assess method accuracy. The laboratory control spikes must show recoveries between 70-130% or the data is rejected. For the results reported here, the laboratory control spikes were within the acceptable range. Laboratory control spike recoveries are given in Attachment B.

4.3 Matrix Spikes

One matrix spike was prepared by adding a known concentration of the target analyte to a sample. Matrix spikes are used to assess method accuracy in the matrix. The matrix spikes should show recoveries between 70-130%. For the results reported here, the matrix spike was within the acceptable range with the exceptions of:

L19345-2 (Deer # 7 3.5 yr male serum) Spk C at 1000 ng/mL for PFOS, which gave low recovery of 68%.

4.4 Laboratory Duplicates

One sample was prepared in duplicate and analyzed. Duplicate results are given along with the sample results in Attachment B.

5 Data Summary

Please see Attachment B for a detailed listing of the analytical results. For the serum samples the results are reported in parts per billion (ng/mL) on an as-received basis.

6 Data/Sample Retention

Samples are disposed of 60 days after the report is issued unless otherwise specified by the project manager. All electronic data is archived on retrievable media and hard copy reports are stored in data folders maintained by MPI Research. Hardcopy data is stored for a minimum of five years. The client will be notified 30 days prior to the disposal of hardcopy data.

7 Attachments

7.1 Attachment A: Chain of Custody

7.2 Attachment B: Analytical Results

7.3 Attachment C: Raw Analytical Data for Water

8 Signatures

Mark Neeley, Research Chemist Associate II — Date: 11-6-09

Robert Zhu, Manager, Analytical — Date: 11/9/09

A

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54943 North Main Street
Mattawan, MI 49071-9399
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(269) 668-4151 Fax

State College
3058 Research Drive
State College, PA 16801
(814) 272-1039 Phone
(814) 231-1580 Fax

Login

Login Group: L0019345

Login #:	19459	Conform COC Sample:	True
Project:	P0005195	Conform COC:	True
Company Name:	Dalton Utilities	Conform Sample:	True
Submitted By:	Dena Haverland	Conform Request:	True
Login Type:	Immediate Receipt of Samples		
Started:	True		
Date Start:	10/27/2009		
Due Date:	11/06/2009		
Login Initiated:	10/27/2009		
Received By:	Ammerman, Mark		
Spread Sample:			
Label:			
MPI SD/PI:	Zhu, Xiang		
Project Title/Type:	PFOA and PFOS Analysis of Serum Samples By LC/MS/MS / ROUTINE		
Login Notes:			

Packages / Containers

Package	Carton	Date / Condition	Shipper / ID	Temp. Control/Temp.	Direction / Handled By
K0022041		Received Date: 10/6/09 10:25 Package & Contents Uncompromised	FEDEX 8694 2057 8178	Dry Ice -79.2	RECEIVED Ammerman, Mark

Container #	Gross Weight	pH	Container Type	Preservative	Mfg. Lot	Mfg. ID
C0457604	3.10 g		2 ml clear plst vial	NONE		
C0457605	3.10 g		2 ml clear plst vial	NONE		
C0457606	3.20 g		2 ml clear plst vial	NONE		
C0457607	3.10 g		2 ml clear plst vial	NONE		
C0457608	3.10 g		2 ml clear plst vial	NONE		
C0457609	3.10 g		2 ml clear plst vial	NONE		
C0457610	3.20 g		2 ml clear plst vial	NONE		
C0457611	3.40 g		2 ml clear plst vial	NONE		
C0457612	3.10 g		2 ml clear plst vial	NONE		
C0457613	3.10 g		2 ml clear plst vial	NONE		
C0457614	3.10 g		2 ml clear plst vial	NONE		
C0457615	3.20 g		2 ml clear plst vial	NONE		
C0457616	3.10 g		2 ml clear plst vial	NONE		
C0457617	3.10 g		2 ml clear plst vial	NONE		
C0457618	3.10 g		2 ml clear plst vial	NONE		
C0457619	3.10 g		2 ml clear plst vial	NONE		
C0457620	3.10 g		2 ml clear plst vial	NONE		
C0457621	3.10 g		2 ml clear plst vial	NONE		
C0457622	3.20 g		2 ml clear plst vial	NONE		
C0457623	3.10 g		2 ml clear plst vial	NONE		

Login

Samples

Sample ID	Container	Matrix	System	System Matrix	Sample	Date Sampled	Date Due
L0019345-0001	C0457604	LIQUID	Deer	Serum	Deer #6 0.5 yr female-serum	10/02/2009	11/06/20
	C0457605						
	C0457606						
	C0457607						
	C0457608						
	C0457609						
	C0457610						
	C0457611						
	C0457612						
	C0457613						
L0019345-0002	C0457614	LIQUID	Deer	Serum	Deer #7 3.5 yr male-serum	10/02/2009	11/06/2009
	C0457615						
	C0457616						
	C0457617						
	C0457618						
	C0457619						
	C0457620						
	C0457621						
	C0457622						
	C0457623						

Login Reviewed By: _____ Date/Time: 10/28/09 1440

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Sample Submittal

Please fax this form before sending samples.

Please send samples to shipping and receiving:
3048 Research Drive, State College, PA 16801
T: (814) 272-1039 • F: (814) 272-1019

MPI Research Contact: Daniel Wright

Send Report To:

Company: Dalton Utilities
Address: 1200 VD Parrott JR Parkway, PO Box 869
City, State, ZIP: Dalton, GA 30722-0869
Attention: Dena Haverland
Phone #: 706-529-1010
Fax #: 706-529-1271
Email: dhaverland@dutil.com
Study/Job #:
Signature/Date:
Printed Name:

Turnaround time (TAT) requirements:

Results Due Date: 30 days
Preliminary Results Format: Verbal (Email) Fax
Report Due Date: 30 days

Storage Conditions

Room temperature
Refrigerator
(Freezer)
Ultra Low freezer
Desiccated
Lighting required
Stability (°C/%RH):
Stability time period:

Safety Information

Special handling:
MSDS attached
Controlled substance:
HAZARDS:
Please fill in the diamond HMIS/NFPA (0 4) if appropriate

	Client ID# Description	Lot/ Control #	Amt. Sent/ Weight	# of Bottles	Matrix	Date & Time	Tests Requested
1	Deer #6 0.5 yr female-Serum		10ml	10	deer	10/2/09 1:08AM	PFOA/PFOS
2	Deer #6 0.5yr female-muscle		as requested	1 bag	deer	10/2/09 2:28AM	PFOA/PFOS
3	Deer #6 0.5yr female-Liver		Whole	1 bag	deer	10/2/09 2:30AM	PFOA/PFOS
4	Deer #7 3.5yr Male-Serum		10ml	10	deer	10/2/09 1:45AM	PFOA/PFOS
5	Deer #7 3.5yr Male-muscle		as requested	1 bag	deer	10/2/09 2:45AM	PFOA/PFOS
6	Deer #7 3.5yr Male-Liver		Whole	1 bag	deer	10/2/09 2:48AM	PFOA/PFOS
7							
8							
9							
10							

PO #:

Notes:

Relinquished by	Date	Time	Received by	Date	Time
Darrell Kavanaugh	10/5/09	6:30pm		10/6/09	1021

V0003836.2

Administrative Form

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TEMPORARY SAMPLE STORAGE FORM

To be completed during ExyLIMS Login

Project #: P5195

Login #: L19345

Initials / Date: MA 10/27/09

One form to be completed for each package

Date / Time Received: 10/26/09 1025

Received By: Mark Ammerman

Shipper: FedEx

Shipper Package ID: 869420577178

Temperature (deg C) / Thermometer ID: -71.2 ID000175

Temperature Control Method: dry ice

Temporary Storage Location: freezer 32

Condition of sample(s):

✓ Good – Package and contents uncompromised
___ Fair – Package damaged / contents uncompromised
___ Poor – Package and contents compromised

Notes:

FedEx Express *US Airbill*

FedEx Tracking Number 8694 2057 8178

Recipient's Copy

1 From

Date 10/5/09 [redacted]

Sender's Name Darrell Kavanagh Phone 706 546-5637

Company USDA Wildlife Services / Wildlife ...

Address 200 Phoenix Road Dept./Floor/Suite/Room

City Athens State GA ZIP 30602

2 Your Internal Billing Reference

3 To

Recipient's Name Daniel Wright Phone 914 [illegible]-1027

Company MPI Research Labs

Recipient's Address 3048 Research Drive Dept./Floor/Suite/Room

We cannot deliver to P.O. boxes or P.O. ZIP codes.

Address

To request a package be held at a specific FedEx location, print FedEx address here.

City State College State PA ZIP 16801

8694 2057 8178

4a Express Package Service — *Packages up to 150 lbs.*

- [X] FedEx Priority Overnight — Next business morning.* Friday shipments will be delivered on Monday unless SATURDAY Delivery is selected.
- [ ] FedEx Standard Overnight — Next business afternoon.* Saturday Delivery NOT available.
- [ ] FedEx First Overnight — Earliest next business morning delivery to select locations.* Saturday Delivery NOT available.
- [ ] FedEx 2Day — Second business day.* Thursday shipments will be delivered on Monday unless SATURDAY Delivery is selected.
- [ ] FedEx Express Saver — Third business day.* Saturday Delivery NOT available.

FedEx Envelope rate not available. Minimum charge: One-pound rate.

* To most locations.

4b Express Freight Service — *Packages over 150 lbs.*

- [ ] FedEx 1Day Freight* — Next business day.** Friday shipments will be delivered on Monday unless SATURDAY Delivery is selected.
- [ ] FedEx 2Day Freight — Second business day.** Thursday shipments will be delivered on Monday unless SATURDAY Delivery is selected.
- [ ] FedEx 3Day Freight — Third business day.** Saturday Delivery NOT available.

* Call for Confirmation:

** To most locations.

5 Packaging

- [ ] FedEx Envelope*
- [ ] FedEx Pak* — Includes FedEx Small Pak, FedEx Large Pak, and FedEx Sturdy Pak.
- [ ] FedEx Box
- [ ] FedEx Tube
- [X] Other

* Declared value limit $500.

6 Special Handling — Include FedEx address in Section 3.

- [ ] SATURDAY Delivery — Not available for FedEx Standard Overnight, FedEx First Overnight, FedEx Express Saver, or FedEx 3Day Freight.
- [ ] HOLD Weekday at FedEx Location — Not available for FedEx First Overnight.
- [ ] HOLD Saturday at FedEx Location — Available ONLY for FedEx Priority Overnight and FedEx 2Day to select locations.

Does this shipment contain dangerous goods? One box must be checked.

- [X] No
- [ ] Yes — As per attached Shipper's Declaration.
- [ ] Yes — Shipper's Declaration not required.
- [X] Dry Ice — Dry Ice, 9, UN 1845 1 x 2.5 kg
- [ ] Cargo Aircraft Only

Dangerous goods (including dry ice) cannot be shipped in FedEx packaging.

7 Payment *Bill to:* Enter FedEx Acct. No. or Credit Card No. below.

- [X] Sender — Acct. No. in Section 1 will be billed.
- [ ] Recipient
- [ ] Third Party
- [ ] Credit Card
- [ ] Cash/Check
- [ ] Obtain Recip. Acct. No.

[redacted]

Total Packages	Total Weight	Total Declared Value†	
1		$ .00	Credit Card Auth.

†Our liability is limited to $100 unless you declare a higher value. See back for details.

8 Residential Delivery Signature Options If you require a signature, check Direct or Indirect.

- [X] No Signature Required — Package may be left without obtaining a signature for delivery.
- [ ] Direct Signature — Someone at recipient's address may sign for delivery. *Fee applies.*
- [ ] Indirect Signature — If no one is available at recipient's address, someone at a neighboring address may sign for delivery. *Fee applies.*

520

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Analytical Report

Summary of Fluorochemical Residues in Serum Samples

	PFOA Perfluorooctanoic Acid	PFOS Perfluorooctanesulfonate
Sample ID	Analyte Found (ng/mL, ppb)	Analyte Found (ng/mL, ppb)
Deer # 7 3.5 yr male-serum	NQ	670^
Deer # 7 3.5 yr male-serum*	NQ	671^
Deer # 6 0.5 yr female-serum	ND	113

*Laboratory Duplicate
ND = Not detected = Response is below the LOD of 1.0 ng/mL (ppb).
NQ = Not quantifiable = Response is between the LOD and the LOQ of 10 ng/mL (ppb).

^ The corresponding Matrix Spike recovery was outside the acceptance criteria of 70-130%, therefore the sample values should be considered an estimate.

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Recovery Summary of Fluorochemical Residues in Serum Samples

Sample Description	PFOA Amount Spiked (ng/mL)	PFOA Amt Found in Sample (ng/mL)	PFOA Amount Recovered (ng/mL)	PFOA Recovery (%)	PFOS Amt Found in Sample (ng/mL)	PFOS Amount Recovered (ng/mL)	PFOS Recovery (%)
LCS A (Data set 110309A) 10 ng/mL	10	ND	10.1	101	ND	9.99	100
LCS B (Data set 110309A) 50 ng/mL	50	ND	47.7	95	ND	47.9	96
LCS A (Data set 110509A) 10 ng/mL	10	N/A	N/A	N/A	ND	10.5	105
LCS B (Data set 110509A) 50 ng/mL	50	N/A	N/A	N/A	ND	49.2	98
Deer # 7 3.5 yr male-serum (L19345-2 Spk C, 50 ng/mL Lab Spike)	50	NQ	51.0	102	670	**	**
Deer # 7 3.5 yr male-serum (L19345-2 Spk C, 1000 ng/mL Lab Spike)	1000	N/A	N/A	N/A	670	1350	68*

ND = Not detected = Response is below the LOD of 1.0 ng/mL.
NQ = Not quantifiable = Response is between the LOD and the LOQ of 10 ng/mL.

* The Matrix Spike recovery was outside the acceptance criteria of 70-130%, therefore the sample values should be considered an estimate.
** The endogenous level of PFOS in the sample significantly exceeds the spiking level, therefore an accurate recovery cannot be calculated.